DEVELOPMENT OF LIMITING DILUTION VIABILITY PCR METHOD TO ASSESS THE EFFECTIVENESS OF SELECTED BIOCIDES TO TREAT INDOOR FUNGI GROWTH

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ABSTRACT

Indoor fungal contamination should be treated with cost-effective and green methods. Biocides have direct biological effect on living organisms but the evidence on their control of indoor fungal contamination is scarce. Using conventional cultivation to evaluate their effectiveness is time consuming while polymerase chain reaction (PCR) provides a fast and reliable alternative. The incorporation of serial dilution technique and viability information in PCR has made it suitable to evaluate the effectiveness of biocides. Thus, this study aimed to assess the antifungal ability of biocides, zinc salicylate (ZS), calcium benzoate (CB) and potassium sorbate (KS) to treat indoor fungal contamination through developing limiting dilution viability PCR (vPCR). These biocides were selected as they successfully controlled the growth of indoor waterborne fungi previously. Indoor air sampling revealed that higher educational building of computer studies (Building A) and of civil engineering studies (Building B) were contaminated by 509 CFU/m^3 and 805.7 CFU/m^3 of indoor airborne fungi, respectively. Two indoor fungi, *Talaromyces* spp. and *Aspergillus niger* were identified. They were subjected to biocides-treatment and subsequent conventional cultivation and limiting dilution vPCR due to their potential risks against humans' health. The limiting dilution vPCR was developed by incorporating the pre-treatment of propidium monoazide (PMA) before deoxyribonucleic acid (DNA) extraction and the serial dilution of the DNA template in PCR. This approach was proven to effectively enumerate the effectiveness of biocides to treat indoor fungi. KS was shown to have the best effectiveness (100%) to prevent the growth of *Talaromyces* spp, followed by ZS (80.8%) and CB (no effect). KS also showed the best effectiveness against *A. niger* (100%) at the early stage of the study but its effect reduced with time. ZS showed durable effect (66.67%) against *A. niger* Day 9 cultures. Inconstant results were indicated by cultivation method. This study has provided a cheaper, more accurate and suitable approach to determine the effectiveness of treatment of indoor fungi than cultivation methods.

ABSTRAK

Pencemaran kulat dalaman perlu dirawat dengan kaedah kos efektif dan mesra alam. Biosida berkesan langsung terhadap organisma tetapi bukti mereka dalam pemulihan pencemaran kulat dalaman berkekurangan. Penilaian kecekapan mereka dengan teknik pengkulturan konvensional sangat mengambil masa. Reaksi rantai polimerase (PCR) menyediakan alternatif yang cepat dan boleh dipercayai. Penggabungan teknik pencairan bersiri dan maklumat untuk mengenal pasti kehidupan kulat dalam PCR menjadikannya lebih sesuai untuk menilai keberkesanan biosida. Jadi, kajian ini bertujuan menilai keupayaan antikulat biosida, iaitu salisilat zink (ZS), kalsium benzoat (CB) dan kalium sorbate (KS) untuk merawat pencemaran kulat dalaman melalui perkembangan *limiting dilution viability-PCR (vPCR)*. ZS, CB dan KS dipilih kerena telah dibuktikan berkesan terhadap kulat berasaskan air dalaman. Pensampelan udara dalaman mendedahkan bahawa bangunan pendidikan tinggi pengajaran komputer (Bangunan A) dan kejuruteraan awam (Bangunan B) tercemar oleh 509 CFU/m^3 dan 805.7 CFU/m^3 kulat udara dalaman masing-masing. Dua kulat dalaman, *Talaromyces* spp. dan *Aspergillus niger* telah dikenalpasti dan dirawat dengan biosida kerana berisiko merbahaya terhadap kesihatan manusia. Pengkulturan konvensional dan *limiting dilution vPCR* digunakan seterusnya untuk menilai kecekapan biosida. *Limiting dilution vPCR* telah diwujudkan dengan memasukkan pra-rawatan propidium monoazide (PMA) sebelum pengekstrakan asid deoksiribonukleik (DNA) dan pencairan bersiri DNA dalam PCR. Pendekatan ini terbukti berkesan untuk menilai kecekapan biosida untuk merawat kulat dalaman. KS ditunjukkan berkecekapan terbaik (100%) untuk mencegah pertumbuhan *Talaromyces* spp, diikuti oleh ZS (80.8%) dan CB (tiada kesan). KS juga menunjukkan kecekapan terbaik terhadap *A. niger* (100%) pada peringkat awal kajian tetapi kesannya menurun dengan masa. ZS menunjukkan kecekapan 66.67% terhadap kultur *A. niger* Hari-9. Keputusan yang tidak sejajar telah ditunjukkan oleh kaedah pengkulturan konvensional. Kajian ini telah menyediakan pendekatan (*limiting dilution vPCR*) yang lebih murah, tepat dan sesuai untuk menentukan kecekapan rawatan kulat dalaman berbandingan kaedah pengkulturan.

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>	more than
%	percent
&	and
<	smaller than
×	times
µg	microgram
µl	microliter
µM	microMolar
A. niger	*Aspergillus niger*
AGE	agarose gel electrophoresis
AIDS	acquired immune deficiency syndrome
ANOVA	Analysis of variance
ASHRAE	American Society for Heating, Refrigerating and Air- Conditioning Engineers
b.p.	base pair
BLAST	Basic Local Alignment Search Tool
BRI	building related illnesses
C1	complained prayer room
C2	complained lecturer room 146
CB	calcium benzoate
CFU	colony forming unit
cm^2	square centimeter
dH_2O	sterile distilled water
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DOSH	Department of Occupational Safety and Health
EMA	ethidium monoazide

EPA	Environmental Protection Agency
et al.	and others
EtBr	ethidium bromide
FPR	female prayer room
g	gram
HVAC	heating, ventilation and air conditioning
i.e.	that is
IAQ	indoor air quality
ICOP-IAQ	industry code of practice on indoor air quality
IEQ	indoor environmental quality
ITS	internal transcribed spacer
JKPPU	Research Management Committees of UTHM
KS	potassium sorbate
L	Litre
m^3	cubic meter
Max	maximum
MEA	malt extract agar
MEB	malt extract broth
mg	miligrams
Mg^{2+}	magnesium ion
Min	minimum
min	minutes
mL	milliliter
mM	miliMolar
mm	millimeter
MPR	male prayer room
MVAC	mechanical ventilating and air conditioning
NaCl	sodium chloride
NC1	non-complained lecturer rooms' lobby
NC2	non-complained chemical engineering laboratory
NCBI	National Center for Biotechnology Information
NIOSH	National Institute of Occupational Safety and Health
NMAM	NIOSH Manual Analytical Method
NYCOSH	New York Committee for Occupational Safety and Health

℃	degree Celsius
PCR	polymerase chain reaction
PMA	propidium monoazide
rDNA	ribosomal deoxyribonucleic acid
RNA	ribonucleic acid
RNase	Ribonuclease
rpm	rotation per minute
rRNA	ribosomal ribonucleic acid
s	seconds
SBS	sick building syndrome
spp.	species
SVOCs	semi volatile organic compounds
TAE	tris-acetate-EDTA
TSA	Trypcase Soy Agar
U	Unit
US	United States
UTHM	Universiti Tun Hussin Onn Malaysia
UV	ultraviolet
V	volt
VOCs	volatile organic compounds
vPCR	viability polymerase chain reaction
W	watt
w/v	weight per volume
ZS	zinc salicylate

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Indoor environmental quality (IEQ) and indoor air quality (IAQ) are very important to health since most of the civilized people stay indoor, where air pollution can be far more severe than outdoor nowadays, most of the time (Orwell *et al*., 2004). The IAQ in any building can be compromised by indoor air pollutants such as microbial contaminants (fungi, bacteria), chemicals (such as carbon monoxide, formaldehyde), allergens, or any mass or energy stressor that can induce health effects. Indoor fungal pollution can bring adverse effects to human health. It is caused by hundreds of species of a typical fungi growing indoor when sufficient moisture is available. It has been reported that dampness and moulds observations are associated with building-related health problems (Pitkaranta *et al*., 2008). According to Pasanen, Lappalainen and Pasanen (1996), nearly 10% of people worldwide have fungal allergy. Plenty of studies pointed out that these fungi are able to produce various kinds of mycotoxins which in turn can cause adverse health effects such as asthma, headache, hypersensitive pneumonia, mucous membrane irritation, damage to body systems. However, very few studies have been done to assess indoor fungal contamination in Malaysia and even Southeast Asian countries which share common hot and humid climate (Lian, Inangda & Ramly, 2007; Ismail, Deros & Leman, 2010; Wardah *et al*., 2011; Hussin *et al*., 2011, Sekhar *et al*., 2003; Luksamijarulkul *et al*., 2009). This is a critical issue as many experts have concluded that the fungal distribution and composition is geographically patterned which make the assessment is a must at every location to ensure the safety of indoor occupants and users (Amend *et al*.,

2010). To the best of the author's knowledge, the studies of indoor fungi contamination in the higher educational building in Malaysia had not been studied before. Hence, the aim of this study is to develop a method to control the growth of specific indoor fungi species found in the higher educational buildings in Malaysia.

Researchers showed that cleaning efforts or remediation were effective in the removal of the fungal problem contributed by indoor mould growth sources (Pitkaranta *et al*., 2011). Some studies have shown that mould exposure and related health symptoms can be reduced by removing the underlying cause of water accumulation and mould growth (Lee *et al*., 2006). However, normal cleaning approaches cannot provide effective and sustainable solution to the fungal contamination as Pitkaranta *et al*. (2011) suggested that fungal particles aerosolized and spread during remediation. Furthermore, continuing replacing the damaged building materials is neither cost-effective nor environmental friendly. Moreover, the normal remediation approaches are not practical in Malaysia because of the high humidity contents in the weather throughout the years. Hence, there is a significant need for a sustainable, low cost and environmental friendly approach to address the IAQ problems. Researchers have sought many different methods of remediation to solve the indoor microbial contamination. Chakravarty and Korva (2013) have found that the growth of fungi was significantly inhibited by five types of antifungal agents (Sanimaster, hydrogen peroxide, isopropyl alcohol, bleach, and Sporicidin) but their effects reduced with time. This phenomenon suggests that the effects of these chemicals are not long-lasting. Moreover, treating the microbes with synthetic fungicides is harmful to the environment and human beings (Verma *et al.*, 2008).

Biocides which are rich in bioactive compounds are safer and more environment-friendly alternatives for indoor fungi control. They have long been used as antimicrobial agents especially in food and pharmaceutical industry. It has given us an insight to apply these substances to treat the indoor microbial contamination as Bellotti *et al.* (2013) has reported that some biocides from food industry, zinc salicylate, calcium benzoate and potassium sorbate, can be used to control the growth of *Chaetomium globosum* and *Alternaria alternate* in indoor waterborne coatings. However, the usage of these natural compounds in the realm of indoor environment and IAQ has not been given full address yet. The indoor fungi isolated from the contaminated higher educational buildings in this study, *Aspergillus niger* (an indoor airborne fungus) and *Talaromyces* spp. (an indoor surface-growing fungus) had not

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